Examining the Correlation Between C-reactive protein Concentration and Glycemic Variability in Adults with Type 2 Diabetes

By

Rebecca Lane

Honors Essay
Department of Nutrition
University of North Carolina at Chapel Hill

Approved:

Advisor: Elizabeth Mayer-Davis

Reader: Laura Young

ABSTRACT

Rebecca Lane: Examining the Correlation Between C-reactive protein Concentration and Glycemic Variability in Adults with Type 2 Diabetes

Purpose:

The purpose of this study was to assess the potential association between glycemic variability (GV) as assessed by standard deviation (SD) of mean blood glucose based on continuous glucose monitoring (CGM) and C-reactive protein concentration (CRP) in adults with type 2 diabetes mellitus.

Methods:

This is a post hoc analysis of data that were collected from a prospective randomized controlled trial of patients with insulin-requiring type 2 diabetes at Mountain Diabetes and Endocrine Center in Asheville, NC. CGM data and CRP levels were obtained at baseline from 42 insulin-treated patients with type 2 diabetes. CRP levels were drawn from the study cohort and frozen, then analyzed by HPLC assay, LabCorp, Burlington, NC. All study subjects wore a 72-hour continuous glucose monitor (CGM) (Medtronic iPro, Medtronic Inc, Northridge, CA) at baseline as part of the study and the standard deviation of blood glucose levels was obtained for each subject from the CGM downloads. The standard deviation was used as a measure of glycemic variability. Data from a total of 39 subjects were used for analysis in this project. All patients had T2DM and were adults above the age of 18 years.

Results:

Mean CRP level was 5.81 (SD, 6.05) mg/L. Mean GV was 50.47 (15.46) mg/dL (n=38). There was no correlation between GV and CRP (r= -.045, NS) after linear regression and correlation

analysis in the total cohort. There was still no association after adjusting for confounding variables (p=0.47, NS).

Conclusion:

Statistical analysis revealed no relationship between GV and CRP in the total cohort of adults with insulin-requiring T2DM.

This original research involved the analysis of data obtained from patients at the Mountain Diabetes and Endocrine Center in Asheville, NC. This research was made possible through data collected by the Mountain Diabetes and Endocrine Center staff members Wendy S. Lane, MD and Chris B. Hale, MA. I would like to thank advisor and mentors Elizabeth J. Mayer-Davis, PhD, Joan Thomas, MS, RD, and Victor Zhong at the University of North Carolina at Chapel Hill, without whose constructive advice, support, and statistical analysis assistance this research would not have been possible. This project was supported by the Tom and Elizabeth Long Excellence Fund for Honors, administered by Honors Carolina.

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LIST OF ABBREVIATIONS

T2DM: Type 2 Diabetes Mellitus

CRP: C-reactive protein

GV: Glycemic variability

ADA: American Diabetes Association

SD: Standard Deviation

CGM: Continuous Glucose Monitor

ASA: Aspirin

CVD: Cardiovascular disease

AHA: American Heart Association

MI: Myocardial Infarction

CHAPTER ONE

INTRODUCTION AND SPECIFIC AIMS

Introduction

Large landmark clinical trials have shown that hyperglycemia contributes to diabetes-related complications, specifically microvascular and macrovascular damage.[12] However, few studies have addressed the correlation between glycemic variability, defined as the troughs and peaks of blood glucose, and the risk of cardiovascular disease. Glycemic variability encompasses acute glucose fluctuations from peaks to nadirs.[3] It has been shown that glycemic variability leads to increased oxidative stress, measured by the oxidative stress marker, 8-iso PGF2alpha.[4] Oxidative stress leads to endothelial dysfunction and inflammation.[5] C-reactive protein (CRP), a protein produced by the liver that has been associated with cardiovascular risk, is released in response to endothelial dysfunction and inflammation.[6] It has been shown that hyperglycemia is a risk factor for cardiovascular disease[7], which raises inflammatory markers such as interleukin-6 and CRP.[8] It has also been shown that there is a correlation between glycemic variability (GV) and the presence of coronary artery disease.[9,10] However, research that specifically examines the association between glycemic variability and coronary artery disease is limited[11] (**Figure 1**).

Epidemiology

According to the U.S. Department of Health and Human Services (DHHS), about 25.8 million people (8.3% of US population) are currently affected by diabetes, 18.8 million people of whom have been diagnosed and 7 million people undiagnosed. In 2010, 25.6 million people ages

20 years or older had diabetes (corresponding to 11.3% of the adult population aged 20 years or older), with 10.9 million people ages 65 or older having diabetes (26.9% of the adult population aged 65 or older). Of those 20 years or older, 13.0 million men (11.8%) had diabetes in 2010, while 12.6 million women (10.8%) were afflicted. It is estimated that 1.9 million people ages 20 years or older were newly diagnosed with diabetes in 2010. People at highest risk of type 2 diabetes include those 45 years or older, those who are overweight or obese, those with diabetic relatives, and those of African American, Alaska Native, American Indian, Asian American, Hispanic/Latino, or Pacific Islander American descent.[12] Heart disease rates are approximately 2-4 times higher in adults with diabetes than in those without,[13] as diabetes itself is a risk factor for cardiovascular disease.[14] Risk factors for CVD among those with diabetes is the same as among those without, however these risk factors tend to be present more often in people with type 2 diabetes than in those without,[15] making type 2 diabetes a risk factor for CVD. Risk factors for heart disease include some factors that cannot be changed such as family history, ethnicity, and age. Risk factors that can be changed include tobacco exposure, hypertension, hypercholesterolemia, obesity, sedentary lifestyle, diabetes, unhealthy diets, and harmful use of alcohol.[16] A 2003 article by the American Heart Association (AHA) concluded that CRP is likely to be one of the best risk markers to examine when measuring risk of CVD.[17] Other markers of CVD risk include adhesion molecules, cytokines, acute-phase reactants other than CRP such as fibrinogen and serum amyloid A (SAA), white blood cell (WBC) count, and erythrocyte sedimentation rate. However some of these markers may not be useful without additional information.[18]

Cost of Diabetes

Diabetes is an expensive disease. According to the American Diabetes Association (ADA), there has been a 41% increase in diabetes costs from 2007-2012. The total cost of diagnosed diabetes in 2007 was estimated to be $174 billion, rising to an estimated $245 billion in 2012.[19] The ADA estimates costs to reach nearly $14,000 per year for people with diagnosed diabetes. These expenditures are estimated to be 2-3 times higher than those of non-diabetic patients.[20] The costs of diabetes are not limited to supplies needed to treat the disease itself (such as blood glucose meters, testing strips and lancets, insulin pumps, insulin, etc.), but also to treat the complications of the disease. These complications are described below and include neuropathy, retinopathy, and nephropathy. Severe acute blood glucose excursions, both hypoglycemic and hyperglycemic, may necessitate emergency intervention or hospitalization, contributing to the financial burden of diabetes on the healthcare system. Because having diabetes invariably engenders additional treatment and associated costs for several diseases beyond just the diabetes itself, it is especially important that physicians and patients alike take appropriate steps in reducing the incidence of diabetes and the severity of its complications.

The purpose of this project is to determine if there is a correlation between CRP concentration and GV in patients with insulin-requiring type 2 diabetes. A positive correlation between CRP and GV, this correlation would lend support to the hypothesis that GV in patients with type 2 diabetes may play a role in inflammation and cardiovascular risk.

Type 2 diabetes mellitus (T2DM)

In adults, Type 2 diabetes mellitus (T2DM) accounts for approximately 90-95% of all diagnosed cases[21] and is the most common form of diabetes today. In this form of diabetes, both impaired insulin secretion and insulin resistance exist. Insulin is a hormone produced by the beta cells of the pancreas that lowers the blood glucose by promoting glucose uptake and utilization in its target tissues, primarily skeletal muscle and fat, where glucose is either utilized as a source of energy or stored as glycogen. T2DM is characterized early in the disease state by insulin resistance, in which the body is unable to properly utilize insulin that is released from the pancreas. In the patient with T2DM, insulin resistance is coupled with impaired insulin production as the beta cells eventually lose the ability to synthesize and secrete insulin.[22] T2DM is strongly associated with obesity, which initially causes insulin resistance and later, through inflammation, induces beta cell injury and loss.[23]

Diabetes complications

T2DM can lead to multiple complications that may increase the difficulty of managing the disease and decrease overall quality of life. These complications include hypertension, diabetic retinopathy (damage to the retina in the eyes that may cause vision impairment or vision loss), nephropathy (kidney disease) that may lead to the need for dialysis or kidney transplantation, neuropathy (damage to peripheral nerves), autonomic neuropathy (damage to nerves of the autonomic nervous system (ANS)), and peripheral vascular disease that may lead to amputations, depression, and in extreme cases, death.[24] Diabetes can also lead to macrovascular complications such as myocardial infarction (MI), and stroke.[25]

Importance of glycemic variability

Recent research suggests that GV may play an important role in the development of cardiovascular complications through increased oxidative stress.[26,27,28,29,30] Oxidative stress is known to contribute to atherosclerosis and heart failure.[31] The mechanism by which glycemic variability is thought to increase oxidative stress that may lead to atherogenesis will be discussed in further detail in chapter 2.

Measures of glycemic variability

Reviewed by David Rodbard, there are several ways to measure glycemic variability.[32] Some of the most common ways to measure GV include percentages such as 'percentage in target range' or 'percentage below target range', mean glucose values, standard deviation of all glucose values, the mean amplitude of glycemic excursions (MAGE), which measures the average amplitude of glucose peaks and troughs with a magnitude greater than 1 SD, and glycemic risk assessment diabetes equation (GRADE), which is a new measure of GV and uses an equation to asses GV. SD is considered to be the simple and classical way of measuring GV[33] and will be discussed in further detail in chapter 2.

Importance of association between C-reactive protein and glycemic variability in T2DM

Coronary heart disease, the most common form of heart disease, costs $108.9 billion annually.[34] Currently, about 1 in 4 deaths, or approximately 600,000 deaths per year, are caused by heart disease, making it the leading cause of mortality today in the U.S. for both men and women.[35] Patients with diabetes mellitus are at increased risk of heart disease. People with diabetes also often have other conditions that are considered risk factors for heart disease. Such

conditions include but are not limited to hypertension, hypercholesterolemia, obesity, a sedentary lifestyle, hyperglycemia, and smoking.[36] In 2005-2008, 67% of adults aged 20 years or older and with self-reported diabetes had hypertension, having a blood pressure reading ≥140/90 mm Hg or using pre-prescription medications for hypertension.[37]

Hyperglycemia is associated with increased cardiovascular risk[38] and thus it is important to target hyperglycemia as a treatment goal of diabetes to decrease this risk. Studies have also shown an association between glycemic variability and oxidative stress markers such as 8-iso-PGF2α, and that normalizing blood glucose concentration can decrease risk of cardiovascular disease.[39,40,41,42] Hyperglycemia also has a positive correlation with CRP concentration.[43] **Figure 2** shows how diabetes mellitus can lead to atherogenesis through hyperglycemia, excess free fatty acids, and insulin resistance eventually leading to increased oxidative stress and ultimately atherogenesis.[44]

Oxidative stress can lead to endothelial dysfunction and eventually atherogenesis. GV is a known cause of oxidative stress and endothelial dysfunction[45], and CRP is widely used as a surrogate marker for endothelial inflammation and cardiovascular risk. If there were an association between GV and CRP in patients with T2DM, then GV may be a target of treatment for preventing increasing CV risk in these patients in addition to targeting measures such as HbA1c.

Specific Aims

1) To conduct a literature review regarding the association between glycemic variability and CRP

Research question 1.1: **Does the literature support an association between GV and CRP?**

2) To determine if there is a correlation between glycemic variability (GV) and C-reactive protein concentration (CRP) among adults with insulin-treated type 2 diabetes

Research question 2.1: **Is there an association between GV and CRP?**

CHAPTER TWO

LITERATURE REVIEW

This literature review will examine current research regarding glycemic variability, continuous glucose monitoring, hyperglycemia, and oxidative stress. It will then cover pertinent literature describing connections between these topics and CRP, inflammation, and cardiovascular complications.

Importance of glycemic variability

Oxidative stress is known to contribute to atherosclerosis and heart failure.[46] GV has been thought to increase oxidative stress through increased production of superoxide by the electron transport chain (ETC). Increased production of superoxide leads to a deleterious cascade that includes events such as increased formation of glycation end products and activation of protein kinase C (PKC).[47] This cascade can ultimately lead to endothelial damage followed by vasoconstriction, inflammation, thrombosis, and eventually atherogenesis (**Figure 2**).[48]

How to measure glycemic variability:

There are several ways to measure GV. Most approaches use CGMs, however, there are a few ways to measure GV using non-CGM approaches. One way of measuring GV is SD of HbA1c. A second measure is the average daily risk range (ADRR). HbA1c is a measurement of

the average blood glucose level over the previous three months, while the ADRR is measured using traditional self-monitoring blood glucose (SMBG) data, or fingersticks. Both HbA1c and SMBG values provide few values to work with and for this reason are not used as frequently to calculate GV as CGM approaches.

There are at least twenty different CGM approaches in measuring glycemic variability.[49] One of the more common criteria is total standard deviation (SD or SD_T). SD is the standard deviation of all glucose values available for a given patient, and is considered to be one of the more simple and classical methods to measure glycemic variability. Most software programs measure SD, making SD an easily accessed measure of glycemic variability. A leading expert on GV, David Rodbard M.D. states that when needing to simply measure GV, SD is likely to be one of the best overall measures to use. Rodbard concludes that SD is highly correlated with the other main measures of glycemic variability.[50] CGM outputs include GV measures such as SD and mean amplitude of glycemic excursions (MAGE) and for this reason it is easy to use this data, which encompasses glucose levels captured every five minutes over a period of several days, rather than using SMBG data which only captures specific time points which are usually hours apart. The CGM outputs used for this analysis did not include MAGE values and so only SD was used as a measure of GV.

Continuous Glucose Monitor (CGM)

A continuous glucose monitor (CGM) is a device that continuously measures patients' glucose concentration over a period of time. Glucose levels are measured every five minutes for as long as the device is worn, which is often 72 or 120 hours at a time. The device consists of a sensor, which is a thin wire inserted just under the skin of the abdomen, arm, or buttocks through

a needle. The needle is removed and the wire is left under the skin to measure the glucose levels of the interstitial fluid as an approximation of blood glucose concentration. A transmitter is present to send information (via radio waves) about glucose levels from the sensor to a monitoring device. The transmitter is removed after a given time period (such as three or five days) and uploaded to a computer. A graph of the patients' sugars over the given time period is generated. This CGM output data may be used for diabetes management in making appropriate decisions about food, exercise, or medications.[51]

CGM Mechanism

A CGM measures the magnitude of the electrical charge produced by a chemical reaction that occurs when the sensor is placed under the skin. The sensor is embedded with the glucose-oxidase enzyme, which catalyzes the reaction of glucose + H_2O → gluconic acid + H_2O_2.[52] The electrode oxidizes hydrogen peroxide into $2H^+ + O_2 + 2e^-$. The electrical gradient that is produced by this charge correlates the interstitial glucose levels into a sensor signal, and this current is transmitted to a processor cable, which analyzes the incoming data and displays the concentrations on the monitor (**Figure 3**).[53] **Figure 4**[54] shows the components of a CGM system.

CGM Limitations

While a CGM is beneficial in the complete picture that it provides, it does have its limitations. For starters, it measures interstitial fluid glucose levels rather than actual blood glucose levels. The sensor also needs to be changed every few days as it gets worn down, and proteins become embedded on it and thereby prevent proper diffusion of glucose to the glucose-oxidase layer. The sensor also needs a warming period in which it acclimates to the environment

of the interstitial fluid before it can function properly. Lastly, the precision of CGMs are dependent upon calibration. The user must perform 2-4 calibrations per day. To calibrate, patients take a fingerstick and enter the blood glucose level from the meter into the sensor to tell the sensor what the meter says. The sensor then uses this information as its own baseline. Calibration can be affected by lag-time, user accuracy, and inherent imprecision of glucose meters. If calibration is done while glycemia is fluctuating, the blood and interstitial glucose concentrations do not correlate. An individual's motivation to calibrate correctly also affects CGM accuracy. People sometimes also become "calibration-happy" in which they over-calibrate. When this happens, the patient is telling the sensor one thing when the sensor may be detecting another, which can lead to sensor miscalculations and inaccuracy. There is also some inherent imprecision of the capillary blood glucose meters used in calibration.

How does hyperglycemia contribute to oxidative stress?

Hyperglycemia induces overproduction of superoxide by the mitochondrial electron transport chain (ETC). Superoxide overproduction is accompanied by an increase in nitric oxide (NO) generation by NO synthase (eNOS) and inducible NO synthase (iNOS). This process favors DNA damage, which is a stimulus for the activation of the nuclear enzyme poly(ADP-Ribose) Polymerase (PARP). PARP depletes the intracellular concentration of its substrate NAD^+. NAD^+ depletion slows the rate of glycolysis, ETC, and ATP formation and produces glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The production of GAPDH results in endothelial dysfunction and contributes to the development of cardiovascular disease (CVD).[55]

What is C-reactive protein?

C-reactive protein (CRP) is a protein in the pentraxin family, a group of proteins recognized for their pentagonal structure and which serve immunological roles.[56] CRP is synthesized by the liver and adipose tissue and is secreted in response to inflammation. It is a non-specific protein[57,58], meaning that its concentration will rise with any sort of acute or chronic inflammation. For this reason CRP is not diagnostic of any specific disease but rather serves solely as an index of acute inflammation or an inflammatory disease occurring in the body.

CRP levels as predictors of CV risk are generally defined as follows:[59]

Normal: CRP <1.00 mg/L

Moderate Risk for CVD: CRP 1.00-3.00 mg/L

High Risk for CVD: CRP>3.00 mg/L

Inflammation and cardiovascular complications

A buildup of plaque in the arterial walls, usually a result of a high intake of low-density lipoprotein (LDL) cholesterol, is often what leads to cardiovascular disease. A buildup of plaque in the arterial wall narrows the arterial lumen, preventing adequate blood flow to the heart. This is known as atherosclerosis, which causes MI. Arterial plaque triggers an immune response, leading to plaque rupture and thrombosis. These events, when they occur in the coronary or cerebral vasculature, result in MI or stroke, respectively.[60] **Figure 2** shows the relationship between inflammation and atherogenesis.[61]

Mechanism by which inflammation leads to CRP elevation

The acute inflammatory response (for example, during infection) stimulates the release of cytokines such as interleukin-6 (IL-6). These cytokines stimulate the liver to synthesize and secrete CRP. CRP binds to phosphocholine, a molecule that is expressed on the outside of damaged cells. This binding activates the complement system, which induces phagocytosis by macrophages. In this way, CRP serves to help remove necrotic cells. CRP concentration may rise above normal within 2-6 hours of acute insult, and tend to peak at 48 hours. The half-life of CRP is approximately 19 hours.[62]

Studies supporting the association of hyperglycemia, GV, and oxidative stress

While more studies have been conducted showing the relationship between hyperglycemia and oxidative stress, some studies have also shown the relationship between glycemic variability and oxidative stress. In a 2008 study, (*Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients)* Ceriello et al. show in an RCT that oscillating glucose between 5 and 15 mmol/L intermittently over 24 hours resulted in significantly more endothelial dysfunction and oxidative stress compared with continuous glucose at either 10 or 15 mmol/L for 24 hours. The authors helped to establish the significance of studying glycemic variability in addition to hyperglycemia as a cause of increased oxidative stress.[63] A 2006 study by Monnier et al. showed MAGE, a measure of GV, correlated with urinary oxidative stress marker 8-iso-PGF2α ($r=0.86, p<.001$). However, the oxidative stress marker did not correlate with HbA1c or other markers of mean glucose exposure. Monnier et al. concluded that oxidative stress was affected more significantly by GV than by chronic sustained hyperglycemia.[64] The same author published

a review article on the subject of GV and oxidative stress in which he concludes that GV ought to be a target of treatment for patients with T2DM.[65]

Other studies have examined not just the relationship between GV and oxidative stress, but also the relationship between GV and cardiovascular complications. In one study, Flaviani et al. found a positive correlation between CONGA-2, a measure of GV, and 8-iso-PGF2α ($r=0.57$, $p=.003$). This oxidative stress marker was found to have an effect on left ventricular mass index (LVMI) ($r=0.42, p=0.031$). There was a significant correlation between GV and 8-iso-PGF2α, suggesting a possible connection between GV, increased activation of oxidative stress, and cardiovascular complications.[66] In a 2009 article published in *Diabetes/Metabolism Research and Reviews*, researchers concluded that GV may play a role in the development of CVD.[67] These studies have shown that there appears to be a correlation between GV and 8-iso-PGF2α. Furthermore, studies have also shown the association between 8-iso-PGF2α and cardiovascular complications.

In a 2004 matched case-control study, researchers found that 8-iso-PGF2α concentration was higher in patients with CHD (n=93) than in patients without (n=93) (8-iso-PGF2α range 120-193 pmol/mmol creatinine for cases compared to 77-139 pmol/mmol creatinine for controls, $p<.001$). Researchers concluded that 8-iso-PGF2α is an independent risk marker of CHD.[68] 8-iso-PGF2α was also found to correlate with CRP concentration, as shown in a 2006 observational study that noticed oxidative stress levels increased with increasing inflammation ($p<.05$) among renal graft patients.[69] Finally, A 2006 review article concluded that CRP is a risk marker of CVD.[70] This article is just one of several that establishes CRP as a marker of CVD.

Putting it all together

In an important 2011 observational study of 344 subjects, Su et al. found Gensini score (used to predict severity of CAD) correlated closely with MAGE ($p<.001$). The researchers also found MAGE to be significantly higher in patients with CAD than in those without ($p<.001$), as well as CRP to be significantly higher in patients with CAD than in those without ($p<.001$). Researchers concluded that MAGE $\geq$3.4 mmol/L is an independent predictor of CAD.[71] These same overarching results were also found in a 2012 study, with CAD patients having higher MAGE levels ($p=.003$) and CRP concentrations ($p=.005$) than patients without CAD. The authors concluded that MAGE and CRP independently predicted CAD.[72] These results were confirmed by a 2013 study in which MAGE and CRP concentration were observed in a group of controls (no T2DM or CAD), a group of patients with T2DM and no CAD, and a group of patients with T2DM and CAD. Researchers found that compared with controls, subjects with T2DM and no CAD had significantly higher MAGE ($p<.001$) and CRP ($p<.05$) concentration. Subjects with T2DM and CAD had significantly higher MAGE levels ($p<.01$) and CRP concentrations ($p<.01$) than subjects with T2DM and no CAD. This study did not report MAGE in relation to CRP.[73] Finally, an important study published in *The New England Journal of Medicine* in 2004 found CRP to be only a moderate risk marker of CVD.[74] However, several limitations of this study warrant careful consideration as there were confounding factors affecting the study population and an extremely long follow-up period; it is thought by the authors and others that these issues may have attenuated the association between CRP and CVD.

CHAPTER THREE

MANUSCRIPT

Abstract

Purpose:

The purpose of this study was to assess the potential association between glycemic variability (GV) as assessed by standard deviation (SD) of mean blood glucose based on continuous glucose monitoring (CGM) and C-reactive protein concentration (CRP) in adults with type 2 diabetes mellitus.

Methods:

This is a post hoc analysis of data that were collected from a prospective randomized controlled trial of patients with insulin-requiring type 2 diabetes at Mountain Diabetes and Endocrine Center in Asheville, NC. CGM data and CRP levels were obtained at baseline from 42 insulin-treated patients with type 2 diabetes. CRP levels were drawn from the study cohort and frozen, then analyzed by HPLC assay, LabCorp, Burlington, NC. All study subjects wore a 72-hour continuous glucose monitor (CGM) (Medtronic iPro, Medtronic Inc, Northridge, CA) at baseline as part of the study and the standard deviation of blood glucose levels was obtained for each subject from the CGM downloads. The standard deviation was used as a measure of glycemic variability. Data from a total of 39 subjects were used for analysis in this project. All patients had T2DM and were adults above the age of 18 years.

Results:

Mean CRP level was 5.81 (SD, 6.05) mg/L. Mean GV was 50.47 (15.46) mg/dL (n=38). There was no correlation between GV and CRP (r= -.045, NS) after linear regression and correlation analysis in the total cohort. There was still no association after adjusting for confounding variables (p=0.47, NS).

Conclusion:

Statistical analysis revealed no relationship between GV and CRP in the total cohort of adults with insulin-requiring T2DM.

Introduction

Research has shown associations between hyperglycemia and cardiovascular disease (CVD), hyperglycemia and CRP, and GV and CVD. However, little research has been conducted regarding whether there is an association between GV and CRP (**Figure 1**).

The purpose of this study was to examine the association between glycemic variability and CRP concentration in patients with type 2 diabetes. Studies have shown that sustained hyperglycemia contributes to increased oxidative stress.[75] Whether GV, too, leads to increased oxidative stress and ultimately heart disease is a topic of current research. The purpose of this paper is to examine the effects of GV on risk of heart disease as measured by CRP.

It is hypothesized that there will be a positive correlation between glycemic variability and the cardiovascular risk marker, C-reactive protein. While causality cannot be established (that is, that glycemic variability itself causes elevation of CRP concentration) in this analysis, it is theoretically feasible that glycemic variability increases oxidative stress, which leads to endothelial dysfunction and inflammation, which in turn could increase CRP.

Materials and Methods

Study Objective

This was a post-hoc, cross-sectional analysis of data collected as part of a prospective randomized controlled trial of a drug combination for use in combination with intensive (basal/bolus) insulin therapy as a treatment option for T2DM. Patients were randomized to one of two groups. Patients in group 1 added the new study drug to their current diabetes regimen, while patients in group 2 did not. The purpose of this study was to test the theory that this study

drug, in conjunction with insulin, would aid both glycemic control and weight loss (or reducing weight gain) in patients with T2DM that take ≥100 units of insulin per day.

Sample

This study population included patients recruited for the trial at the Mountain Diabetes and Endocrine Center in Asheville, North Carolina. Per study protocol, all subjects had been diagnosed with T2DM for at least twelve months (mean duration of disease, 17 (7) years). Study subjects had HbA1c values 6.5-9% with mean A1c= 7.9%. The study population included both genders; most (95.2%) were white. Study subjects were all treated with intensive basal/bolus insulin regimens with or without additional oral or injectable glucose-lowering agents.

Study Criteria

Inclusion criteria were: age between 18-80, presence of T2DM ≥1 year, on intensive (basal/bolus) insulin therapy and taking ≥100 units/day. Exclusion criteria were: pregnancy, history of pancreatitis, thyroid cancer, or Multiple Endocrine Neoplasia (MEN) syndrome, recent (within previous six months) serious chronic illness, a recent acute coronary event, or recent significant use of glucocorticoids (>2 week usage in the previous 3 months). Patients were instructed to be fasting at baseline.

Blood Sampling

Fasting blood samples were shipped ambient within 12 hours. Blood measures included CRP in addition to several other tests including a comprehensive metabolic blood profile, complete blood count (CBC) and differential, TSH, and a complete lipid profile. A CBC is a

panel of 14 blood tests that broadly measure kidney and liver function as well as electrolyte and fluid balance.[76] CRP concentration was measured by immunochemiluminometric assay (ICMA).

Continuous Glucose Monitor (CGM)

The CGM that was used for this study was the Medtronic iPro CGM (Northridge, CA). The iPro CGM sensor was inserted subcutaneously on the abdomen and the device was worn on three separate occasions for 72 hours at a time. The iPro measured glucose concentration at five minute intervals during the 72 hours, although patients were blinded to the CGM readings. The device was removed 72 hours later and uploaded to obtain the patient's glucose profile. The CGM download listed number of sensor values, average daily and 3-day blood glucose values, minimum and maximum glucose values, SD, number of meter values, average meter value, minimum/maximum meter values, optimal accuracy criteria including number of paired readings, mean absolute difference (MAD%), correlation coefficient, number of high and low excursions, duration above, within, and below limits, pie charts for each duration, glucose area above and below limit, and average blood glucose value per each time period. The SD of the baseline CGM downloads were obtained and used as the measure of GV in this analysis. Subjects' data were excluded in the case of missing or incomplete information such as a patient not wearing the CGM for 72 hours.

Statistical Analysis

SAS 9.3 software (SAS Institute, Cary, North Carolina) was used to analyze all data. Values are presented in tables as mean (SD) or value (percentage). To test for the association between GV and CRP, we used correlation analysis and linear regression models. CRP was log-

transformed due to a highly skewed distribution. Assumptions of the linear regression were examined to see if they were violated, including the linearity of the relationship between exposure (glycemic variability) and outcome (Log transformed CRP), homoscedasticity of the errors, and normality of the error distribution. Assumptions were met for all linear models. We conducted a formal test of interaction to measure for effect measure modification (EMM), choosing $p \leq 0.2$ as a cut point for an effect measure modifier. We also adjusted for potential confounders such as age, A1c, BMI, aspirin, and % bolus to basal. After obtaining baseline characteristics (**tables 1 and 2**), the unadjusted association of CRP with GV was evaluated (**table 3**). We then adjusted for age and gender (**table 4**), then further adjusted for A1c and BMI (**table 5**), then further adjusted for statin use, aspirin use, and % bolus to basal (**table 6**). Stratification analyses were also conducted (**table 7**). A p value ≤ 0.05 determined statistical significance.

Results

The mean age of all study subjects (n=42) was 58 (11) years, with 21 subjects under 60 years of age and 21 subjects over 60 years of age. Of the original 42 subjects, 17 (40.5%) were male and 25 (59.5%) were female. Fifteen subjects (35.7%) were fasting at baseline. Twenty-four subjects (57.1%) had A1c $\leq$ 7.8% at baseline and 18 subjects (42.9%) had A1c >7.8%. Twenty subjects (47.6%) were on statins at baseline and 22 subjects (52.4%) were not using statins. Eighteen subjects (42.9%) were taking aspirin (ASA) at baseline, and 24 subjects (57.1%) were not using ASA. Twenty-two subjects (52.4%) had BMI $\leq$40 kg/m^2 and 18 patients (42.9%) had BMI >40 kg/m^2 (range 31.8-81.3 kg/m^2) (**Table 1**).

After testing for EMM, gender and statin use were found to be effect measure modifiers (p-values = 0.1934 and 0.0829, respectively). We then calculated stratum-specific estimates. However, the results were not biologically plausible likely due to small sample size or unmeasured confounding. It was found that with every 1 unit increase in SD_T in males, there is a 1.02 unit increase in CRP levels, and with every 1 unit increase in SD_T in females, there is a 12.28 unit increase in CRP levels. Among those not using statins, with every 1 unit increase in SD_T, there is a 0.98 unit decrease in CRP levels, and among those using statins with every 1 unit increase in SD_T, there is a 0.099 unit decrease in CRP levels. These results are not biologically plausible and for this reason gender and statin use were used as confounding variables, as discarding the variables in the model is not rational due to their potential association with exposure and outcome.

Mean CRP value was 5.81(6.05) mg/L (n=39). Mean GV was 50.47 (15.46) mg/dL (n=38). In the total cohort, there was no correlation between GV and CRP after linear regression and correlation analysis (r= -.045, NS).

Table 7 shows mean CRP according to tertiles of CRP and tertiles of GV, and **Table 8** shows mean GV according to tertiles of CRP and tertiles of GV. Linear regression output models for crude data showed no association between GV and CRP in the total cohort (p=0.7870, NS) (**Table 3**). Association remained non-significant after adjusting for confounding variables (**Tables 4-6**).

Discussion

No relationship was observed between GV and CRP in the total cohort of insulin-using type 2 diabetic subjects. There may be no association between GV and CRP.

It may be that CRP is too non-specific to correlate with GV. It is possible that other inflammatory markers may correlate more closely with GV and less with other inflammatory conditions occurring in older adults. A marker more specific to endothelial inflammation and oxidative stress, such as 8-iso-PGF2α, may have proven a better correlate with GV than CRP in this patient population. Another potential explanation for the lack of correlation between GV and CRP in this study may be the measurement of GV. SD was used to calculate GV; it is possible that another measure of GV such as MAGE may have proven to be a better correlate with GV. MAGE accounts for major fluctuations in glucose levels that are greater than 1 SD and does not account for minor fluctuations; thus MAGE may have yielded a better correlation with CRP than did SD by only incorporating major, ie, clinically significant, glucose fluctuations. The CGM data that were obtained for this study did not include MAGE values and so for this reason MAGE was not used in data analysis. However, it has been shown that there is a strong correlation between MAGE and SD (r=0.89),[77] so it is unclear whether using MAGE would actually have yielded significantly different results from SD.

CRP as a marker of cardiovascular risk has several shortcomings, particularly in this population. There is a strong correlation between cardiovascular disease and obesity[78] and CRP is a marker for both. As a non-specific inflammatory marker, any source of inflammation will raise CRP concentration. Adipose tissue is inflammatory, and several studies have shown a correlation between obesity and CRP concentration.[79,80,81] Studies have shown that CRP and GV independently predict CVD,[82] but there is a paucity of literature describing a direct correlation between CRP and GV. Zhang et al did describe a correlation between CRP and GV in a cohort of subjects with T2DM.[83] Our findings differ from those, but our study population may have also

differed in that ours was a more obese, insulin-using American population with more comorbidities.

Two important limitations relate to the patients' fasting status and duration of diabetes. Although patients were instructed to be fasting at baseline, fewer than half actually were. Although fasting status was controlled for, it may be that some other behavioral factors related to patients' non-fasting status confound the association between GV and CRP. Regarding duration of diabetes, this factor may be associated with both inflammation and glycemic control and as such may be a confounder. However, although all patients had diabetes duration $\geq$1 year, exact duration was not available so adjustment for potential confounding was not possible.

This study also had a very limited sample size. Accuracy increases with increasing sample size, and it may be that our sample size was too small to appropriately assess whether there is a correlation between CRP and GV in patients with T2DM. It is also sometimes thought that studies conducted by a single center are subjected to the same biases.

We also only had 3 days' worth of CGM data. These particular 3 days for patients may have been an 'off' three days as they may have been specifically eating more appropriately now that they had put on CGM devices. It may be that this variability did not adequately represent what patients normally eat on a daily basis and that the variability is not an accurate value compared to what it would normally be. It may have been more accurate to use CGM and CRP data from towards the end of the study when patients would likely either have permanently changed their eating habits or resorted back to old ones so that the GV measure more accurately represents the norm for these patients.

Lastly, these patients also required a large amount of insulin each day. It is possible that insulin itself confounded the relationship between GV and CRP.

This cohort comprised a chronically ill obese study population with many inflammatory comorbidities including but not limited to periodontal disease, arthritis, infection, and occult malignancy. These conditions may have been more powerful contributors to CRP concentration than GV, precluding the ability to accurately define any relationship between CRP and GV.

Implications

CRP measures CVD risk in individuals regardless of other chronic illnesses, however patients with T2DM tend to have higher basal levels of CRP than individuals without T2DM.[84,85] Although CRP and GV are both considered to be independent markers for cardiovascular risk in patients with T2DM, there was no correlation between the two even when controlling for other variables (gender, A1c, statin use, concomitant medications, BMI, age). This suggests that CRP may be affected by other concomitant inflammatory conditions besides glycemic variability in this high-risk population. Because this population is frequently affected by such inflammatory conditions as arthritis, periodontal disease, infections, as well as obesity itself, all of which may elevate CRP, it is possible that these inflammatory comorbidities may have had greater effects on CRP than glycemic parameters, including absolute magnitude of hyperglycemia (HbA1c) or the more subtle glucose fluctuations (GV). CRP therefore may be too non-specific for use as a marker of cardiovascular risk this patient population.

In conclusion, more research needs to be done regarding the association between CRP and GV in patients with T2DM to accurately define the association between the two, if any. Future studies should be larger and include individuals across a spectrum of BMI ranging from normal to obese as opposed to only obese individuals with multiple comorbidities. Additional markers of GV (such as MAGE) and of inflammation (such as IL-6) and oxidative stress (such as 8-iso-PGF2α) should also be evaluated. Measuring MAGE in addition to SD may lead to a

correlation between GV and CRP, as MAGE has been found to correlate with CRP in previous studies.[86,87] Lastly, evaluating a patient cohort with a lower mean baseline CRP level may reveal better correlations between measures of glycemic variability and cardiovascular risk markers. Future studies should possibly be directed at studying a patient population with lower BMIs and fewer comorbidities, and use a more specific marker of endothelial dysfunction such as 8-iso-PGF2α to better define the relationship between GV and CV risk.

Table 1. GV and CRP by demographic characteristics

	N	GV*, mean (SD)	P value	CRP, mean (SD)	P value
Gender			0.2		0.04
Male	17	53.76 (17.50)		3.62 (3.57)	
Female	25	47.81 (13.44)		7.51 (7.04)	
Age			0.9		0.9
≤ 60	21	50.29 (13.83)		5.72 (7.80)	
> 60	21	50.62 (17.00)		5.90 (4.23)	
BMI			0.3		0.7
≤ 40	22	52.86 (16.44)		5.45 (7.46)	
> 40	18	47.53 (14.08)		6.29 (3.65)	
Fasting			0.4		0.5
No	26	48.46 (11.79)		5.14 (3.80)	
Yes	15	53.54 (21.13)		6.41 (8.68)	
A1c			0.9		0.2
≤ 7.8	24	50.22 (15.86)		4.83 (3.44)	
> 7.8	18	50.87 (15.37)		7.23 (8.47)	
Pump			0.9		0.3
No	22	50.71 (11.59)		6.60 (7.36)	
Yes	15	50.07 (20.69)		4.53 (3.84)	
Group			0.6		0.9
1	24	49.33 (15.11)		5.89 (7.28)	
2	18	51.88 (16.23)		5.72 (4.15)	
Statin			0.2		0.07
No	22	46.78 (10.88)		7.64 (7.74)	
Yes	20	53.80 (18.30)		4.09 (3.16)	
ASA			0.2		0.5
No	24	47.86 (14.40)		5.23 (3.68)	
Yes	18	54.06 (16.60)		6.58 (8.25)	

*GV is the standard deviation of the blood glucose readings from CGM (SDtotal). CRP units are mg/L and GV units are mg/dL.

**Median cutpoints were used for A1c, BMI, and age

Table 2. GV and demographic and clinical characteristics by CRP value

	CRP<3.0, %	CRP >=3.0, %	P value
N	12	27	
GV, mean (sd)	49.9 (13.6)	50.7 (16.5)	0.9
GV, median	47.0	50.0	0.8
Gender			0.001
Male	83.3	25.9	
Female	16.7	74.1	
Age			0.3
≤ 60	58.3	40.7	
> 60	41.7	59.3	
BMI			0.10
≤ 40	75.0	44.4	
> 40	25.0	55.6	
Fasting			1.0
No	66.7	61.5	
Yes	33.3	38.5	
A1c			0.5
≤ 7.8	50.0	63.0	
> 7.8	50.0	37.0	
Pump			0.5
No	50.0	64.0	
Yes	50.0	36.0	
Group			0.7
1	50.0	59.3	
2	50.0	40.7	
Statin			0.7
No	41.7	51.9	
Yes	58.3	48.2	
ASA			0.7
No	50.0	59.3	
Yes	50.0	40.7	

Table 3. Association between GV and CRP in total sample population (crude)

Parameter Estimates		
Variable	**Parameter Estimate**	**Pr > |t|**
Intercept	1.43854	0.0101
SDTotal	-0.00273	0.787

*Interpretation: for every 1 unit increase in SD_T there is a(n) $e^{-.00273}$=0.997 unit increase in CRP

Table 4. Association between GV and CRP in total sample population, adjusting for age, gender, and fasting status

Parameter Estimates		
Variable	**Parameter Estimate**	**Pr > |t|**
Intercept	0.37734	0.6655
SDTotal	0.0023	0.7993
Age	0.00378	0.7493
Gender	1.0239	0.0008
Fasting	-0.09017	0.7589

*Interpretation: Holding all other variables constant, for every 1 unit increase in age, there is a(n) $e^{0.0023}$=1.0023 unit increase in CRP, etc.

Table 5. Association between GV and CRP in total sample population, adjusting for age, gender, fasting status, A1c, and BMI

Parameter Estimates		
Variable	**Parameter Estimate**	**Pr > |t|**
Intercept	-2.34442	0.3832
SDTotal	0.00577	0.5264
Age	0.00537	0.6834
Gender	1.06449	0.0006
Fasting	-0.15735	0.6147
A1c	0.10505	0.664
BMI	0.03985	0.0767

*Interpretation: Holding all other variables constant, for every 1 unit increase in A1c there is a(n) $e^{0.10505}$=1.1107 unit increase in CRP, etc.

Table 6. Association between GV and CRP in total sample population, adjusting for age, gender, fasting status, A1c, BMI, percentage of bolus to basal, statin use, and ASA use

Parameter Estimates		
Variable	**Parameter Estimate**	**Pr > |t|**
Intercept	-2.55102	0.4177
SDTotal	0.00769	0.4659
Age	0.00909	0.5972
Gender	0.94731	0.0099
Fasting	-0.01781	0.9619
A1c	0.1023	0.7162
BMI	0.04256	0.0925
Percentage of Bolus to Basal	0.00135	0.8952
Statin	-0.45951	0.1396
ASA	-0.03927	0.9

*Interpretation: Holding all other variables constant, with every 1 unit increase in percentage of bolus to basal, there is a(n) $e^{0.00135}$= 1.0014 unit increase in CRP, etc.

Table 7. Mean (SD) of CRP according to tertiles of CRP and tertiles of GV

	1st tertile_GV	2nd tertile_GV	3rd tertile_GV
1st tertile_CRP	1.39 (1.31)	1.92 (0.91)	1.80 (0.97)
2nd tertile_CRP	4.43 (0.85)	4.73 (1.09)	4.05 (1.16)
3rd tertile_CRP	10.02 (4.24)	9.31 (2.35)	8.77 (3.47)
Total_CRP	5.45 (4.08)	4.85 (3.73)	4.81 (3.49)

Table 8. Mean (SD) of GV according to tertiles of CRP and tertiles of GV

	Total_GV	1st tertile_GV	2nd tertile_GV	3rd tertile_GV
1st tertile_CRP	53.38 (18.06)	36.33 (5.86)	47.33 (2.66)	75.25 (16.38)
2nd tertile_CRP	48.77 (16.93)	34.33 (9.14)	51.00 (5.66)	65.20 (9.73)
3rd tertile_CRP	49.17 (10.97)	37.00 (8.72)	50.25 (1.26)	60.25 (0.96)

Figure 1. The Open Question of Whether Glycemic Variability is Associated with CRP

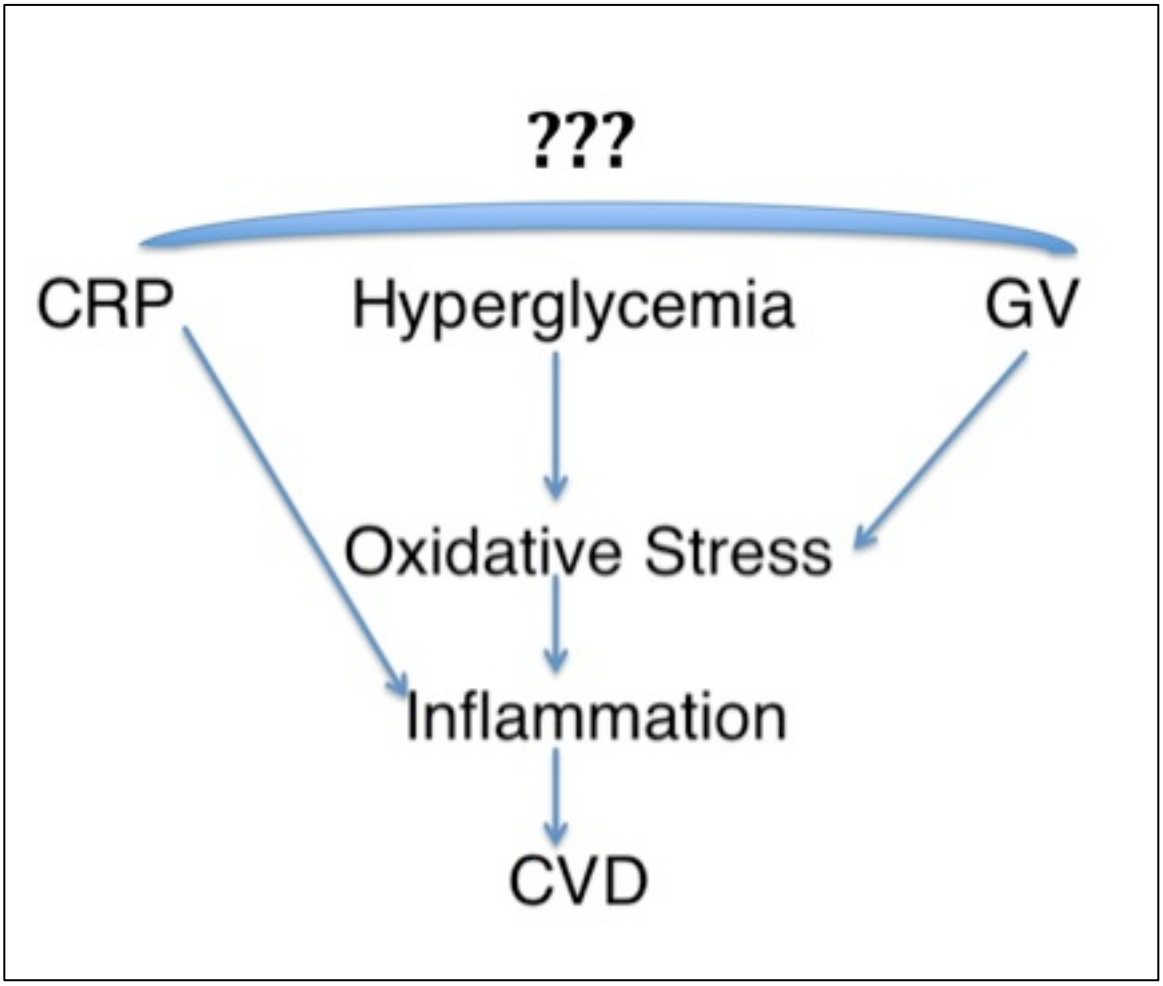

Figure 2. Mechanism by which Diabetes Mellitus Contributes to Atherogenesis[44]

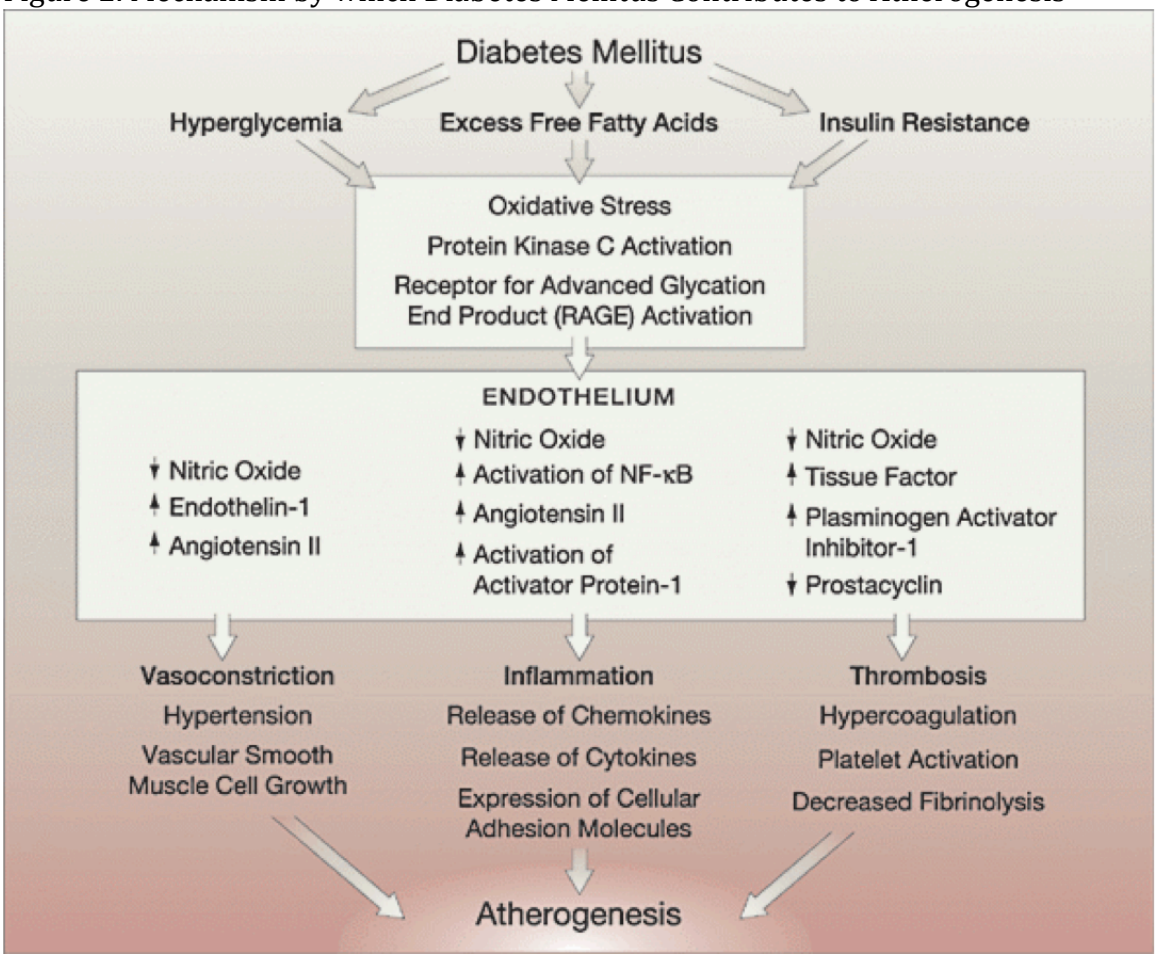

Figure 3. Mechanism of Continuous Glucose Monitoring[53]

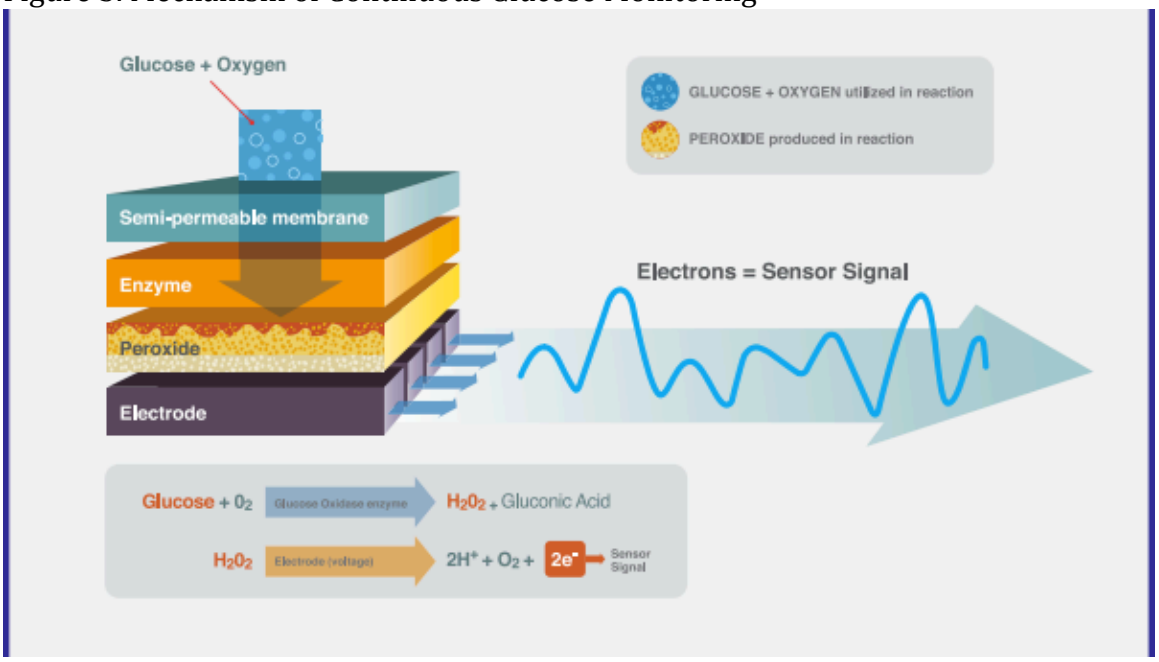

Figure 4. Components of a Continuous Glucose Monitoring System[54]

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APPENDIX

Table 9. CRP and glycemic variability of 39 patients included in correlation analysis

Patient ID	CRP, mg/L	Glycemic Variability*, mg/dL
01	1.28	34
02	5.04	58
03	5.28	24
04	3.30	73
05	7.37	61
06	1.01	82
07	4.39	36
08	6.61	42
09	1.42	52
10	15.63	40
11	5.50	55
12	0.99	48
13	5.80	61
14	12.67	52
15	5.50	22
16	3.21	39
17	6.87	42
18	3.19	78
19	0.13	32
20	0.90	47
21	35.22	**
22	8.15	60
23	2.71	47
24	1.91	59
25	2.75	43
26	3.93	42
27	5.57	61
28	8.55	50
29	3.96	47
30	1.14	65

31	3.16	56
32	8.84	50
33	2.78	44
34	2.70	46
35	10.96	24
36	13.77	59
37	3.13	95
38	7.19	49
39	4.27	43

**GV calculated as SD*
***Data unavailable*

Table 10. Full model showing association between GV and CRP in total sample population (crude)

Parameter Estimates					
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	**1**	1.43854	0.52937	2.72	0.0101
SDTotal	**1**	-0.00273	0.01004	-0.27	0.787

*Interpretation: for every 1 unit increase in SD_T there is a(n) $e^{-.00273}$=0.997 unit increase in CRP

Table 11. Full model showing association between GV and CRP in total sample population, adjusting for age, gender, and fasting status

Parameter Estimates					
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	**1**	0.37734	0.86483	0.44	0.6655
SDTotal	**1**	0.0023	0.00895	0.26	0.7993
Age	**1**	0.00378	0.01171	0.32	0.7493
Gender	**1**	1.0239	0.27526	3.72	0.0008
Fasting	**1**	-0.09017	0.29126	-0.31	0.7589

*Interpretation: Holding all other variables constant, for every 1 unit increase in age, there is a(n) $e^{0.0023}$=1.0023 unit increase in CRP, etc.

Table 12. Full model showing association between GV and CRP in total sample population, adjusting for age, gender, fasting status, A1c, and BMI

Parameter Estimates					
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	**1**	-2.34442	2.649	-0.89	0.3832
SDTotal	**1**	0.00577	0.009	0.64	0.5264
Age	**1**	0.00537	0.01303	0.41	0.6834
Gender	**1**	1.06449	0.27763	3.83	0.0006
Fasting	**1**	-0.15735	0.30931	-0.51	0.6147
A1c	**1**	0.10505	0.23942	0.44	0.664
BMI	**1**	0.03985	0.02174	1.83	0.0767

*Interpretation: Holding all other variables constant, for every 1 unit increase in A1c there is a(n) $e^{0.10505}$=1.1107 unit increase in CRP, etc.

Table 13. Full model showing association between GV and CRP in total sample population, adjusting for age, gender, fasting status, A1c, BMI, percentage of bolus to basal, statin use, and ASA use

Parameter Estimates					
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	**1**	-2.55102	3.09562	-0.82	0.4177
SDTotal	**1**	0.00769	0.01038	0.74	0.4659
Age	**1**	0.00909	0.01699	0.54	0.5972
Gender	**1**	0.94731	0.33952	2.79	0.0099
Fasting	**1**	-0.01781	0.36889	-0.05	0.9619
A1c	**1**	0.1023	0.27823	0.37	0.7162
BMI	**1**	0.04256	0.02433	1.75	0.0925
Percentage of Bolus to Basal	**1**	0.00135	0.01013	0.13	0.8952
Statin	**1**	-0.45951	0.30115	-1.53	0.1396
ASA	**1**	-0.03927	0.36705	-0.11	0.9

*Interpretation: Holding all other variables constant, with every 1 unit increase in percentage of bolus to basal, there is a(n) $e^{0.00135}$= 1.0014 unit increase in CRP, etc.